Journal of Basic and Environmental Sciences
ISSN
Online:2356-6388
Print:2536-9202

Research Paper Open Access

Isolation and characterization of multi-drug resistant *Enterococcus faecalis* as a causative agent of dental abscesses and infections in Egyptian patients

Mohamed O. Abdel-Monem[1], Mohamed Khedr[2], Asmaa Ramadan[1] and Noha K. El-Dougdoug[1]

[1] Botany and Microbiology Department, Faculty of Science, Benha University.

[2] Botany and Microbiology Department, Faculty of Science, Al Azhar University, Cairo.

Corresponding author: E-mail address: asmaarm@yahoo.com

Abstract: *Enterococcus faecalis (E. faecalis)* is a gram-positive bacterium that causes dental calculus in humans. It is also the principal pathogen responsible for dental abscesses, which are partly erupted teeth in which bacteria become trapped between the crown and soft tissues, producing pain and inflammation. This study aimed to isolate and characterize antibiotic-resistant bacteria causing dental infection. Microbiological examination was performed on the samples to detect different kinds of bacteria. The isolates were identified morphologically, biochemically using the automated VITEK® 2 system. Isolates identified were *E. faecalis.*

Disk diffusion tests were used to determine the antibiotic sensitivity pattern of isolated dental abscess bacteria. Five isolates proved positive for resistance to most of the antibiotics examined.

Key words: *Enterococcus faecalis (E. faecalis),* dental oral infection, antimicrobial resistance.

1. Introduction

E. faecalis is a Gram-positive bacterium that is facultatively anaerobic and can live in harsh conditions such as high salt concentrations and temperatures above 45°C [1]. *E. faecalis* is a normal

microbiota present in the gastrointestinal system, oral cavity, and vagina of humans and animals [2,3]. Many strains of *E. faecalis* are multidrug resistant and cause a variety of diseases [1]**.** It is difficult to treat the stomach and other infection sites [4]. It causes bacteremia, endocarditis, urinary tract infections, meningitis, wound infections, pelvic and intraabdominal infections, and nosocomial and iatrogenic infections [5,6,7]. In dentistry, *E. faecalis* has been linked to dental caries in chronic periodontitis and persistent in apical periodontitis. [2,8]. Filling root canals are the most obvious source of *E. faecalis* in the oral cavity [9]. *E. faecalis* is one example of a pathogen that is difficult to eliminate in dentistry and is one of the most prevalent causes of root canal treatment failures [10]. Its persistence and survival, as well as its presence as a pathogen in root canals, make it a more essential virulence factor [11]. *E. faecalis* hides from the immune system and antibiotics.

To increase bacterial control before root canal sealing, several antiseptic and antibiotic materials like calcium hydroxide or antibiotic pastes are used. However, *E. faecalis* cells were discovered in root canals after many days of endodontic therapy, even after the use of calcium hydroxide [12,13,14]. *E. faecalis* seems to fit the ecological condition of the root and able to survive in an environment with scant available nutrients [15]. Even at low levels, *E. faecali*s can induce difficult-to-treat infections due to its susceptibility to disinfection procedures during endodontic treatment, particularly when creating a biofilm [16].

Many individuals worldwide die as a result of multidrug resistance [17] it is causes 700,000 global deaths every year, and is expected that the death numbers will rise to 10 million deaths by 2050 [18,19].

In the current study, *E. faecalis that* was isolated previously from patients with dental infections exhibited antibiotic-resistant features.

2. Materials and methods

2.1. Isolation of pathogenic bacteria

Specimens were collected from different patients' men and women during over seven month's period **(April 2021 - October 2021).** Specimens were collected from buccal cavity, saliva and over tooth by swabs. Samples collected

from patients in five private clinics and General Authority for Health Hospital in Giza, Egypt. A total number of 25 patients (15 female and 10 male). The age of the study group ranged from 20 year-65 years. The clinical specimens were immediately transported using brain heart infusion broth medium to the laboratory for bacteriological analysis according to [20].

Primary identification for bacteria

All samples were streaked on bile esculin agar as a selective media for *Enterococcus* isolates.

After 24 hours, the pure cultures were gram stained and viewed under the microscope, after which the pure cultures were inoculated into slants and stored at 4℃ for further identification.

All 25 isolates streaked on blood agar plates to identify their blood hemolysis type**.** According to [21], nutrient agar medium autoclaved for 15 minutes at 121°C and after cooling to 45-50°C, about (7%) sterile blood was added.

Twenty five isolates were selected from the preserved slants for identification using conventional methods described previously [22] as following: Motility test [23], Potassium hydroxide test [24], Catalase activity [25], Slide and tube coagulase test [26], Cytochrome (Oxidase test)[27], Carbohydrate fermentation [28] , Nitrate reduction [29], Indole production test [30], Voges-Proskauer (V-P) test: Glucose- peptone medium [31] and Glucose-salt medium [32]**,** Citrate utilization test Citrate utilization medium [33, Urease production medium [34]

2.2. Automated Identification

Automated identification by using the Biomerieux Vitek 2 system. The VITEK 2 is an automated microbial identification system. With its colorimetric reagent cards, and associated hardware and software advances, the VITEK 2 offers a state-of-the-art technology platform for phenotypic identification methods. The reagent cards have 64 wells that can each contain an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalization, enzyme hydrolysis, and growth in the presence of inhibitory substances. An optically clear film present on both sides of the card allows for the appropriate level of oxygen transmission while maintaining a sealed vessel that prevents contact with the organism-substrate admixtures. Each

card has a pre-inserted transfer tube used for inoculation. Cards have bar codes that contain information on product type, lot number, expiration date, and a unique identifier that can be linked to the sample either before or after loading the card onto the system.

2.3. Antibiotic susceptibility test

Using Kirby-Bauer method [35] antibiotic susceptibility test was carried out on for the five positive *Enterococcus* isolates. In this assay, 10 types of antibiotic disks were used as following Aztreonam (30 µg), Bacitracin (0.04 µg), Clindamycin (2 µg), Fucidic acid (10 µg), imipenem (10 µg), Nalidixic acid (30 µg), Norfloxacin (10 µg), Ofloxacin (5 µg) and Streptomycin (10 µg). vancomycin (30µg). 0.5 McFarland standards of bacteria was used and inoculated and spread by a sterile swab on Muller-Hinton agar Medium. Antibiotic discs were then placed on inoculated agar plates by forceps. The plates were left in incubator upside down at 37 ° C for 18-24 hr.

3. Results and Discussion

3.1. Isolation of pathogenic bacteria

Specimens were taken from various patients, both men and women, that extend a seven-month period **(April 2021 - October 2021).** Specimens were taken from abscess, gingivitis, and General Authority for Health Hospital. There was a total of 25 patients (15 females and 10 males). The research group's age varied from 20 to 65 years **(Fig.1).**

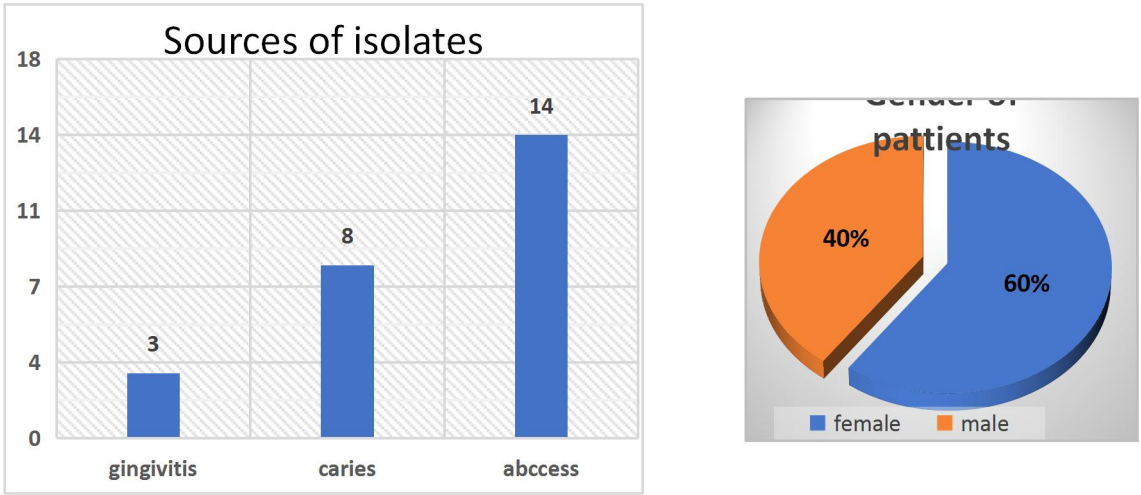

Figure (1): sources of clinical specimens correlated with patient's gender.

3.2. Primary identification

3.2.1. Morphological identification of bacterial isolates

The 25 swabs were suspended in sterile solution buffer 0.85% NaCl tubes and serial dilution was performed to isolate and purify bacterial isolates. About 100 µl of streaked on nutrient agar plates in addition to bile esculin agar plates in triplicate (3plates from each dilution), incubated at 37 °C for 24 hours. Total various bacterial colonies isolated and purified from all dilutions were cultivated on both nutrient agar and bile esculin agar that promise as Enterococci were picked up as a single colony and coded for future investigation. The primary step in identifying the 25 isolates was a gram stain reaction which confirmed that all 25 isolates were gram-positive.

Only five of the 25 bacterial isolates were able to grow on bile esculin agar after an overnight incubation at 37°C (**Fig. 2**)

The selected isolates were tested for their type of hemolysis through blood agar media that exhibit alpha hemolysis **(Fig.3)**

Figure (2): *E. faecalis* on bile esculin agar media

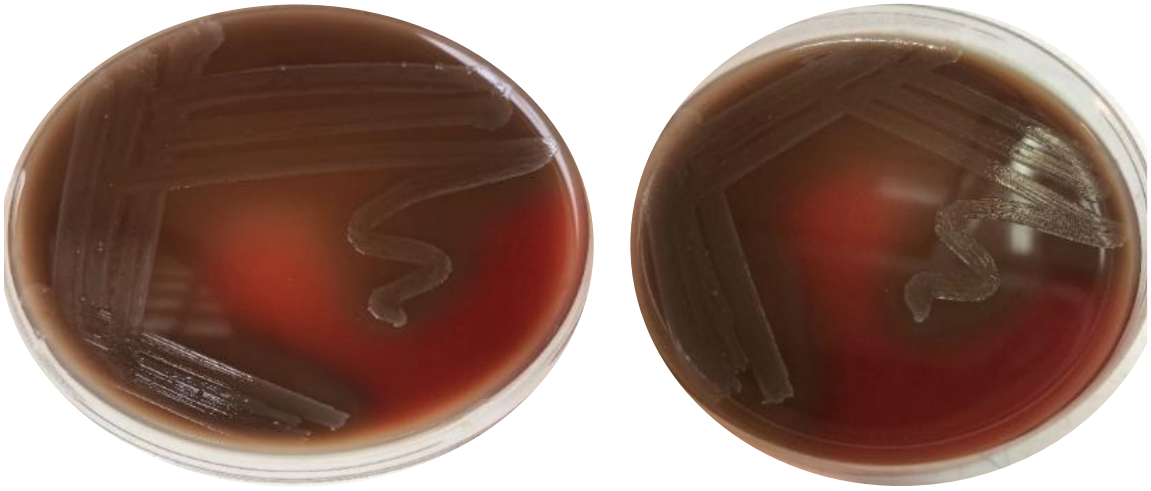

Figure (3) *E. faecalis* with alpha hemolysis on blood agar media.

3.2.2. Biochemical characterization of bacterial isolates

Biochemical characterization of 5 isolates includes eleven tests as listed in **(Table 1).** According to the results, the Multi drug resistant bacteria were *E. faecalis.* Automated identification utilizing the Biomerieux Vitek 2 system validated the identifications.

Table (1): Biochemical characterization of bacterial isolates.

code / Test	*A 1*	*A 2*	*A 3*	*A 4*	*A 5*
Motility	**-Ve**	**-Ve**	**-Ve**	**-Ve**	**-Ve**
Catalase	**-Ve**	**-Ve**	**-Ve**	**-Ve**	**-Ve**
Oxidase	**-Ve**	**-Ve**	**-Ve**	**-Ve**	**-Ve**
glucose Fermentation	**+Ve**	**+Ve**	**+Ve**	**+Ve**	**+Ve**
Nitrate Reduction	**+Ve**	**+Ve**	**+Ve**	**+Ve**	**+Ve**

Vogues Proskour	**+Ve**	**+Ve**	**+Ve**	**+Ve**	**+Ve**
Indole Production	**-Ve**	**-Ve**	**-Ve**	**-Ve**	**-Ve**
Citrate Utilization	**-Ve**	**-Ve**	**-Ve**	**-Ve**	**-Ve**
H2S Production	**-Ve**	**-Ve**	**-Ve**	**-Ve**	**-Ve**
Urease	**-Ve**	**-Ve**	**-Ve**	**-Ve**	**-Ve**
Coagulase	**-Ve**	**-Ve**	**-Ve**	**-Ve**	**-Ve**
Suspected Organism	***E. faecalis***	***E. faecalis***	***E. faecalis***	***E. faecalis***	***E. faecalis***

3.3. Antimicrobial susceptibility testing

Because of (AMR), our capacity to treat common diseases becomes more difficult, resulting in increased disease duration, costs, complications, and death rates. (AMR) is expected to cause 10 million fatalities by 2050 [36], while another research estimates that (AMR) would cost the global economy US$100 trillion during the same time period [37]. In our study the strain was tested against 10 antibiotics and was found to be resistant to at most antibiotics **(Table 2 and Fig.4). A 2** was resistant to the majority of antibiotics used. Antibiotic-resistant *E. faecalis* strains are becoming more common in clinical settings, and the protection provided by biofilm development makes therapies such as antiseptic rinses or antibacterial dressings more useless. To control *E. faecalis*, novel methods must be devised and executed. [38]. The use of lytic bacteriophages is a promising application under evaluation for combating multidrug resistant *E. faecalis* strains and associated biofilms. [39].

Antibiotic resistance can be developed through mutations in chromosomal genes or by mobile genetic elements (horizontally acquired resistance) In that view, a resistance that is acquired through mutation, mechanism of horizontally acquired resistance, or overexpression of the drug efflux were discussed previously [40]

Table (2): Antibiotics zone of growth inhibition (mm)

Clinical isolates	Antibiotics zone of growth inhibition (mm)										
	IPM	S	B	OFX	ATM	FA	DA	NA	NOR	VAN	
A1	0	0	0	10	0	25	0	0	0	0	
A2	30	0	0	22	0	18	0	0	12	0	
A3	27	0	0	20	0	20	0	0	15	0	
A4	30	0	0	20	0	23	0	0	10	0	
A5	30	12	0	17	0	23	0	0	15	0	

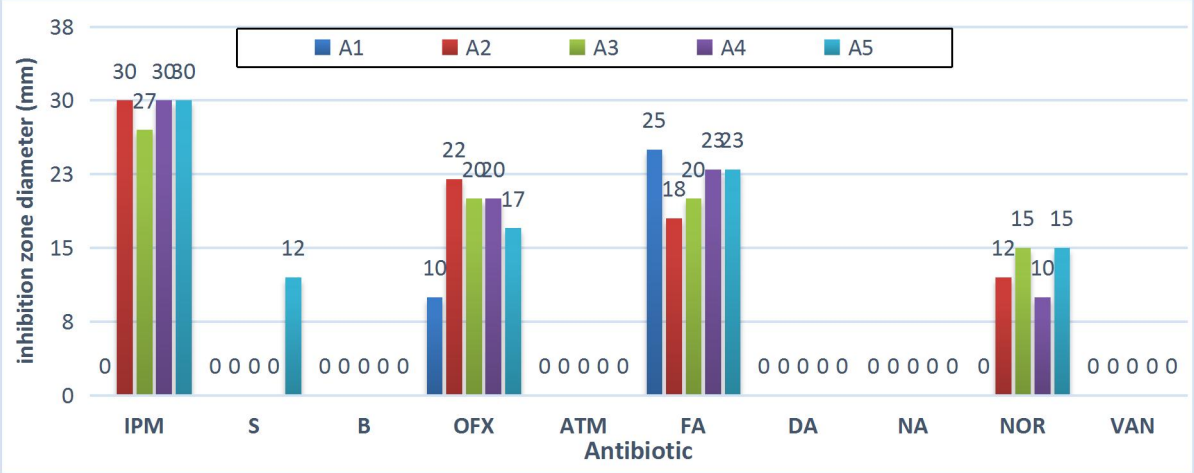

Figure (4): Antibiotic susceptibility test with inhibition zone diameter.

4. References:

1. Pinheiro, E. T., & Mayer, M. P. A. (2014). Enterococcus faecalis in oral infections. *JBR Journal of Interdisciplinary Medicine and Dental Science*, 3(1), 1-5.
2. Mayer, P. E. (2015). *Enterococcus faecalis in Oral Infections. JBR Journal of Interdisciplinary Medicine and Dental Science,* 03(01).
3. Kayaoglu, G., & Ørstavik, D. (2004). Virulence factors of Enterococcus faecalis: relationship to endodontic disease. *Critical Reviews in Oral Biology & Medicine*, *15*(5), 308-320
4. Higuita, N. I. A., & Huycke, M. M. (2014). Enterococcal disease, epidemiology, and implications for treatment. In *Enterococci: From commensals to leading causes of drug resistant infection*. Massachusetts Eye and Ear Infirmary.
5. Teixeira, L. M., Carvalho, M. G. S., Shewmaker, P. L., & Facklam, R. R. (2011). *Enterococcus*. *Manual of clinical microbiology*,11, 403-421.
6. Vu, J., & Carvalho, J. (2011). Enterococcus: review of its physiology, pathogenesis, diseases and the challenges it poses for clinical microbiology. *Frontiers in Biology*, *6*(5), 357.
7. Murray, P. R., Rosenthal, K. S., Kobayashi, G. S., & Pfaller, M. A. (2009). Enterococcus and other gram-positive cocci. *Medical Microbiology*, *3*,40-55.
8. Najafi, K., Ganbarov, K., Gholizadeh, P., Tanomand, A., Rezaee, M. A., Mahmood, S. S., & Kafil, H. S. (2020). Oral cavity infection by *Enterococcus faecalis*: virulence factors and pathogenesis. *Reviews in Medical Microbiology*, *31*(2), 51-60.
9. Cole, M. F., Bryan, S., Evans, M. K., Pearce, C. L., Sheridan, M. J., Sura, P. A., & Bowden, G. H. (1999). Humoral immunity to commensal oral bacteria in human infants: salivary secretory immunoglobulin A antibodies reactive with *Streptococcus mitis* biovar 1, *Streptococcus oralis*, *Streptococcus mutans*, and during the first two years of life. *Infection and immunity*, *67*(4), 1878-1886.
10. Khalifa, L., Shlezinger, M., Beyth, S., Houri-Haddad, Y., Coppenhagen-Glazer, S., Beyth, N., & Hazan, R. (2016). Phage therapy against Enterococcus faecalis in dental root canals. *Journal of oral microbiology*, *8*(1), 32157.
11. Rôças, I. N., Siqueira Jr, J. F., & Santos, K. R. (2004). Association of Enterococcus faecalis with different forms of periradicular diseases. *Journal of endodontics*, *30*(5), 315-320.
12. Vivacqua-Gomes, N., Gurgel-Filho, E. D., Gomes, B. P. F. A., Ferraz, C.C. R., Zaia, A. A., & Souza-Filho, F. J. (2005). Recovery of

Enterococcus faecalis after single‐or multiple‐visit root canal treatments carried out in infected teeth ex vivo. *International Endodontic Journal*, *38*(10), 697-704.

13. Sjögren, U., Figdor, D., Spångberg, L., & Sundqvist, G. (1991). The antimicrobial effect of calcium hydroxide as a short‐term intracanal dressing. *International endodontic journal*, *24*(3), 119-125.
14. Byström, A., & Sundqvist, G. (1981). Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. *European Journal of Oral Sciences*, *89*(4), 321-328.
15. Sundqvist, G., Figdor, D., Persson, S., & Sjögren, U. (1998). Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*, *85*(1), 86-93.
16. Chávez de Paz, L. E., Bergenholtz, G., Dahlén, G., & Svensäter, G. (2007). Response to alkaline stress by root canal bacteria in biofilms. *International endodontic journal*, *40*(5), 344-355.
17. Chanishvili, N. (2012). Phage therapy history from Twort and d'Herelle through Soviet experience to current approaches. *dvances in virus research*. 83, 3-40.
18. Tagliabue, A., & Rappuoli, R. (2018). Changing priorities in vaccinology: antibiotic resistance moving to the top. *Frontiers in immunology*, *9*,1068.
19. O'Neill, J. (2014). AMR Review Paper-Tackling a crisis for the health and wealth of nations. *AMR Review Paper*.

.

20. Miller, J.M. (2005): A guide to specimen management in clinical microbiology. 3th ed. book. *American society for microbiology, United States of America.*
21. Farrell I. D. and Robinson L. (1972). Abortion due *Brucella abortus* in sheep in Nigeria. *J. Appl. Bact*. 35: 625-630.
22. C.H. Collins, P.M. Lyne, J.M. Grange, J.O. Falkinham. (2004). Identification methods, Collins and Lyne's Microbiological Methods, eigth ed., pp. 89–109.
23. Clark W. A., Hollis D. G., Weaver R. E. and Riley P. (1984). Identification of Unusual Pathogenic Gram negative Aerobic and Facultatively Anaerobic Bacteria. Atlanta, Georgia: U.S. Department of Health and Human Services.
24. Shushan, H., Betty, H., Sydney, M. F. and Rial, D.R. (1981). Rapid method that aids in distinguishing Gram positive from Gram negative anaerobic bacteria. *J. Clin. Microbiol*. 13(3): 444-448.
25. Benson (2001). Microbiological Applications (Laboratory Manual in general microbiology) 8th Ed. The Mc Graw-Hill.

26. Vandenesch, F., Naimi, T., Enright, M. C., Lina, G., Nimmo, G. R., Heffernan, H. and Etienne, J. (2003): Community-acquired methicillin-resistant Staphylococcus aureus carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerging infectious diseases, 9(8), 978-984.

27. Kovacs N. (1956). Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature (London)*. 178: 703.

(1948). Studies in the bacteriology of milk. II. The *Staphylococci and Micrococci* of milk. *J. Dairy Res.* 15, 249.

32. Smith N. R., Gorden R. E., and Clark F. E. (1946). Aerobic mesophilic spore forming bacteria. U. S. Dept. Agr. Misc.

33. Simmons J. S. (1926). A culture medium for differentiating organisms of typhoid colon aerogenes groups and for isolation of certain fungi. *J. Infect. Dis*. 39: 209 – 214.

34. Christensen, W. B. (1946). Urea Decomposition as a Means of Differentiating Proteus and Paracolon Cultures from Each Other and from *Salmonella* and *Shigella* Types. *J. Bacteriol. Oct.* 52(4): 461–466.

35. WHO (World Health Organization) (2003). Basic laboratory procedures in clinical bacteriology .2nd ed. Geneva, Switzerland: 2003. pp. 103–121.

36. Trotter, A. J., Aydin, A., Strinden, M. J. and O'Grady, J. (2019). Recent and emerging technologies for the rapid diagnosis of infection and

28. Manual of Microbiological Methods (1957). By the American Society of the Microbiology. Mc Graw Hill Book Co., Inc.

29. Barrow G. I. and Feltham R. K. (1993). Cowan and steel's Manual for the Identification of Medical Bacteria. 2nd ed. Cambridge Univ. Press. London.

30. Atlas R. M. (1993). Hand book of microbiological media. Lawerence C. Parks, CRC Press. Boca KRaton, Ann. Arbor, London, Tokyo.

31. Abd-El-Malek, Y. and Gibson, T. antimicrobial resistance. *Curr. Opin. Microbiol.* 51, 39–45.

37. Neill O. J. (2019). Review on Antimicrobial Resistance. Tackling drug Resistant Infections Globally: Final Report and Recommendations 2016 October 13.

38. Distel, J.W.; Hatton, J.F.; Gillespie, M.J. (2002). Biofilm formation in medicated root canals. J. Endod. 28, 689–693.

39. Khalifa, L.; Brosh, Y.; Gelman, D.; Coppenhagen-Glazer, S.; Beyth, S.; Poradosu-Cohen, R.; Que, Y.A.; Beyth, N.; Hazan, R. (2015). Targeting Enterococcus faecalis biofilms with phage therapy. Appl. Environ. Microbial. 81, 2696–2705.

40. Foster TJ. (2017). Antibiotic resistance in *Staphylococcus aureus*. Current status and future prospects. *FEMS Microbiol Rev*; 41(3):430-449.